

HAPLOTYPES OF THE ICAM2 GENE

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 60/201,946 filed May 5, 2000.

FIELD OF THE INVENTION

This invention relates to variation in genes that encode pharmaceutically-important proteins. In particular, this invention provides genetic variants of the human Intercellular Adhesion Molecule 2 (ICAM2) gene and methods for identifying which variant(s) of this gene is/are possessed by an individual.

BACKGROUND OF THE INVENTION

Current methods for identifying pharmaceuticals to treat disease often start by identifying, cloning, and expressing an important target protein related to the disease. A determination of whether an agonist or antagonist is needed to produce an effect that may benefit a patient with the disease is then made. Then, vast numbers of compounds are screened against the target protein to find new potential drugs. The desired outcome of this process is a lead compound that is specific for the target, thereby reducing the incidence of the undesired side effects usually caused by activity at non-intended targets. The lead compound identified in this screening process then undergoes further *in vitro* and *in vivo* testing to determine its absorption, disposition, metabolism and toxicological profiles. Typically, this testing involves use of cell lines and animal models with limited, if any, genetic diversity.

What this approach fails to consider, however, is that natural genetic variability exists between individuals in any and every population with respect to pharmaceutically-important proteins, including the protein targets of candidate drugs, the enzymes that metabolize these drugs and the proteins whose activity is modulated by such drug targets. Subtle alteration(s) in the primary nucleotide sequence of a gene encoding a pharmaceutically-important protein may be manifested as significant variation in expression, structure and/or function of the protein. Such alterations may explain the relatively high degree of uncertainty inherent in the treatment of individuals with a drug whose design is based upon a single representative example of the target or enzyme(s) involved in metabolizing the drug. For example, it is well-established that some drugs frequently have lower efficacy in some individuals than others, which means such individuals and their physicians must weigh the possible benefit of a larger dosage against a greater risk of side effects. Also, there is significant variation in how well people metabolize drugs and other exogenous chemicals, resulting in substantial interindividual variation in the toxicity and/or efficacy of such exogenous substances (Evans et al., 1999, *Science* 286:487-491). This variability in efficacy or toxicity of a drug in genetically-diverse patients makes many drugs ineffective or even dangerous in certain groups of the population, leading to the failure of such drugs in clinical

trials or their early withdrawal from the market even though they could be highly beneficial for other groups in the population. This problem significantly increases the time and cost of drug discovery and development, which is a matter of great public concern.

It is well-recognized by pharmaceutical scientists that considering the impact of the genetic variability of pharmaceutically-important proteins in the early phases of drug discovery and development is likely to reduce the failure rate of candidate and approved drugs (Marshall A 1997 *Nature Biotech* 15:1249-52; Kleyen PW et al. 1998 *Science* 281: 1820-21; Kola I 1999 *Curr Opin Biotech* 10:589-92; Hill AVS et al. 1999 in *Evolution in Health and Disease* Stearns SS (Ed.) Oxford University Press, New York, pp 62-76; Meyer U.A. 1999 in *Evolution in Health and Disease* Stearns SS (Ed.) Oxford University Press, New York, pp 41-49; Kalow W et al. 1999 *Clin. Pharm. Therap.* 66:445-7; Marshall, E 1999 *Science* 284:406-7; Judson R et al. 2000 *Pharmacogenomics* 1:1-12; Roses AD 2000 *Nature* 405:857-65). However, in practice this has been difficult to do, in large part because of the time and cost required for discovering the amount of genetic variation that exists in the population (Chakravarti A 1998 *Nature Genet* 19:216-7; Wang DG et al 1998 *Science* 280:1077-82; Chakravarti A 1999 *Nat Genet* 21:56-60 (suppl); Stephens JC 1999 *Mol. Diagnosis* 4:309-317; Kwok PY and Gu S 1999 *Mol. Med. Today* 5:538-43; Davidson S 2000 *Nature Biotech* 18:1134-5).

The standard for measuring genetic variation among individuals is the haplotype, which is the ordered combination of polymorphisms in the sequence of each form of a gene that exists in the population. Because haplotypes represent the variation across each form of a gene, they provide a more accurate and reliable measurement of genetic variation than individual polymorphisms. For example, while specific variations in gene sequences have been associated with a particular phenotype such as disease susceptibility (Roses AD *supra*; Ulbrecht M et al. 2000 *Am J Respir Crit Care Med* 161: 469-74) and drug response (Wolfe CR et al. 2000 *BMJ* 320:987-90; Dahl BS 1997 *Acta Psychiatr Scand* 96 (Suppl 391): 14-21), in many other cases an individual polymorphism may be found in a variety of genomic backgrounds, i.e., different haplotypes, and therefore shows no definitive coupling between the polymorphism and the causative site for the phenotype (Clark AG et al. 1998 *Am J Hum Genet* 63:595-612; Ulbrecht M et al. 2000 *supra*; Drysdale et al. 2000 *PNAS* 97:10483-10488). Thus, there is an unmet need in the pharmaceutical industry for information on what haplotypes exist in the population for pharmaceutically-important genes. Such haplotype information would be useful in improving the efficiency and output of several steps in the drug discovery and development process, including target validation, identifying lead compounds, and early phase clinical trials (Marshall et al., *supra*).

One pharmaceutically-important gene for the treatment of HIV infection and inflammatory diseases is the Intercellular Adhesion Molecule 2 (ICAM2) gene or its encoded product. ICAM2 is a cell surface adhesion molecule that is constitutively expressed on the endothelium, and is involved in leukocyte recruitment into tissues (McLaughlin et al., *J. Cell Sci.* 1999; 112 (Pt 24):4695-4703). ICAM2 interacts with the lymphocyte function-associated antigen LFA-1, which acts as a mediator in a wide range of lymphocyte, monocyte, natural killer cell, and granulocyte interactions with other cells in

immunity and inflammation (Butini et al., Eur. J. Immunol. 1994; 24:2191-2195; Staunton et al., Nature 1989; 339:61-64). This interaction is a critical step in syncytium formation induced by human immunodeficiency virus type 1 (HIV-1). Circulating levels of ICAM2 are increased during HIV infection (Galea et al., Res. Immunol. 1997; 148:109-117). Among an HIV-infected population, the circulating ICAM2 levels were higher in the asymptomatic group compared to the AIDS group, whereas circulating ICAM1 levels were higher in the AIDS group. Thus, the authors concluded that serum circulating ICAM2 and ICAM1 in HIV infection could act as markers to discriminate between asymptomatic and progressor patients.

ICAM2 has also been shown to be associated with the etiology of inflammatory disorders (Gerwin et al., Immunity. 1999; 10:9-19). Mice deficient in ICAM2 were shown to have prolonged eosinophil accumulation in the lung during allergic lung inflammation, resulting in extended hyperresponsiveness. The authors suggest that these results reveal an essential role for ICAM2 in the development of the inflammatory and respiratory components of allergic lung disease.

The Intercellular Adhesion Molecule 2 gene is located on chromosome 17q23-q25 and contains 4 exons that encode a 275 amino acid protein. Reference sequences for the ICAM2 gene (Genaissance Reference No. 1396765; SEQ ID NO: 1), coding sequence (GenBank Accession No: NM_000873.1), and protein are shown in Figures 1, 2 and 3, respectively.

There is one known polymorphism in the ICAM2 gene that corresponds to a guanine or adenine at a position corresponding to nucleotide position 79037 in Figure 1 (HGBASE:SNP000008392). Because of the potential for variation in the ICAM2 gene to affect the expression and function of the encoded protein, it would be useful to know whether additional polymorphisms exist in the ICAM2 gene, as well as how such polymorphisms are combined in different copies of the gene. Such information could be applied for studying the biological function of ICAM2 as well as in identifying drugs targeting this protein for the treatment of disorders related to its abnormal expression or function.

SUMMARY OF THE INVENTION

Accordingly, the inventors herein have discovered 11 novel polymorphic sites in the ICAM2 gene. These polymorphic sites (PS) correspond to the following nucleotide positions in Figure 1: 74697 (PS1), 75110 (PS2), 75141 (PS3), 75221 (PS4), 76302 (PS5), 78155 (PS6), 78181 (PS7), 78187 (PS8), 78875 (PS9), 78902 (PS10), and 78961 (PS11). The polymorphisms at these sites are cytosine or guanine at PS1, cytosine or thymine at PS2, adenine or guanine at PS3, thymine or cytosine at PS4, adenine or guanine at PS5, guanine or adenine at PS6, thymine or guanine at PS7, adenine or guanine at PS8, guanine or adenine at PS9, guanine or adenine at PS10, and guanine or adenine at PS11. In addition, the inventors have determined the identity of the alleles at these sites, as well as at the previously identified site at nucleotide position 79037 (PS12), in a human reference population of 79 unrelated individuals self-identified as belonging to one of four major population groups: African descent, Asian, Caucasian and Hispanic/Latino. From this information, the inventors deduced a set of

knowledge as to the phenotypic effect of any ICAM2 haplotype or haplotype pair, apply the information derived from detecting ICAM2 haplotypes in an individual to decide whether modulating ICAM2 activity would be useful in treating the disease.

The claimed invention is also useful in screening for compounds targeting ICAM2 to treat a specific condition or disease predicted to be associated with ICAM2 activity. For example, detecting which of the ICAM2 haplotypes or haplotype pairs disclosed herein are present in individual members of a population with the specific disease of interest enables the pharmaceutical scientist to screen for a compound(s) that displays the highest desired agonist or antagonist activity for each of the most frequent ICAM2 isoforms present in the disease population. Thus, without requiring any *a priori* knowledge of the phenotypic effect of any particular ICAM2 haplotype or haplotype pair, the claimed haplotyping method provides the scientist with a tool to identify lead compounds that are more likely to show efficacy in clinical trials.

The method for haplotyping the ICAM2 gene in an individual is also useful in the design of clinical trials of candidate drugs for treating a specific condition or disease predicted to be associated with ICAM2 activity. For example, instead of randomly assigning patients with the disease of interest to the treatment or control group as is typically done now, determining which of the ICAM2 haplotype(s) disclosed herein are present in individual patients enables the pharmaceutical scientist to distribute ICAM2 haplotypes and/or haplotype pairs evenly to treatment and control groups, thereby reducing the potential for bias in the results that could be introduced by a larger frequency of an ICAM2 haplotype or haplotype pair that had a previously unknown association with response to the drug being studied in the trial. Thus, by practicing the claimed invention, the scientist can more confidently rely on the information learned from the trial, without first determining the phenotypic effect of any ICAM2 haplotype or haplotype pair.

In another embodiment, the invention provides a method for identifying an association between a trait and an ICAM2 genotype, haplotype, or haplotype pair for one or more of the novel polymorphic sites described herein. The method comprises comparing the frequency of the ICAM2 genotype, haplotype, or haplotype pair in a population exhibiting the trait with the frequency of the ICAM2 genotype or haplotype in a reference population. A higher frequency of the ICAM2 genotype, haplotype, or haplotype pair in the trait population than in the reference population indicates the trait is associated with the ICAM2 genotype, haplotype, or haplotype pair. In preferred embodiments, the trait is susceptibility to a disease, severity of a disease, the staging of a disease or response to a drug. In a particularly preferred embodiment, the ICAM2 haplotype is selected from the haplotypes shown in Table 5, or a sub-haplotype thereof. Such methods have applicability in developing diagnostic tests and therapeutic treatments for HIV infection and inflammatory diseases.

In yet another embodiment, the invention provides an isolated polynucleotide comprising a nucleotide sequence which is a polymorphic variant of a reference sequence for the ICAM2 gene or a fragment thereof. The reference sequence comprises SEQ ID NO:1 and the polymorphic variant

comprises at least one polymorphism selected from the group consisting of guanine at PS1, thymine at PS2, guanine at PS3, cytosine at PS4, guanine at PS5, adenine at PS6, guanine at PS7, guanine at PS8, adenine at PS9, adenine at PS10, and adenine at PS11. In a preferred embodiment, the polymorphic variant comprises an additional polymorphism of adenine at PS12.

A particularly preferred polymorphic variant is an isogene of the ICAM2 gene. An ICAM2 isogene of the invention comprises cytosine or guanine at PS1, cytosine or thymine at PS2, adenine or guanine at PS3, thymine or cytosine at PS4, adenine or guanine at PS5, guanine or adenine at PS6, thymine or guanine at PS7, adenine or guanine at PS8, guanine or adenine at PS9, guanine or adenine at PS10, guanine or adenine at PS11 and guanine or adenine at PS12. The invention also provides a collection of ICAM2 isogenes, referred to herein as an ICAM2 genome anthology.

In another embodiment, the invention provides a polynucleotide comprising a polymorphic variant of a reference sequence for an ICAM2 cDNA or a fragment thereof. The reference sequence comprises SEQ ID NO:2 (Fig.2) and the polymorphic cDNA comprises at least one polymorphism selected from the group consisting of thymine at a position corresponding to nucleotide 12, guanine at a position corresponding to nucleotide 43, adenine at a position corresponding to nucleotide 660, adenine at a position corresponding to nucleotide 687, and adenine at a position corresponding to nucleotide 746. In a preferred embodiment, the polymorphic variant comprises an additional polymorphism of adenine at a position corresponding to nucleotide 822. A particularly preferred polymorphic cDNA variant comprises the coding sequence of an ICAM2 isogene defined by haplotypes 1- 2 and 4 - 14.

Polynucleotides complementary to these ICAM2 genomic and cDNA variants are also provided by the invention. It is believed that polymorphic variants of the ICAM2 gene will be useful in studying the expression and function of ICAM2, and in expressing ICAM2 protein for use in screening for candidate drugs to treat diseases related to ICAM2 activity.

In other embodiments, the invention provides a recombinant expression vector comprising one of the polymorphic genomic variants operably linked to expression regulatory elements as well as a recombinant host cell transformed or transfected with the expression vector. The recombinant vector and host cell may be used to express ICAM2 for protein structure analysis and drug binding studies.

In yet another embodiment, the invention provides a polypeptide comprising a polymorphic variant of a reference amino acid sequence for the ICAM2 protein. The reference amino acid sequence comprises SEQ ID NO:3 (Fig.3) and the polymorphic variant comprises at least one variant amino acid selected from the group consisting of alanine at a position corresponding to amino acid position 15 and aspartic acid at a position corresponding to amino acid position 249. A polymorphic variant of ICAM2 is useful in studying the effect of the variation on the biological activity of ICAM2 as well as on the binding affinity of candidate drugs targeting ICAM2 for the treatment of HIV infection and inflammatory diseases.

The present invention also provides antibodies that recognize and bind to the above polymorphic ICAM2 protein variant. Such antibodies can be utilized in a variety of diagnostic and prognostic

formats and therapeutic methods.

The present invention also provides nonhuman transgenic animals comprising one of the ICAM2 polymorphic genomic variants described herein and methods for producing such animals. The transgenic animals are useful for studying expression of the ICAM2 isogenes *in vivo*, for *in vivo* screening and testing of drugs targeted against ICAM2 protein, and for testing the efficacy of therapeutic agents and compounds for HIV infection and inflammatory diseases in a biological system.

The present invention also provides a computer system for storing and displaying polymorphism data determined for the ICAM2 gene. The computer system comprises a computer processing unit; a display; and a database containing the polymorphism data. The polymorphism data includes the polymorphisms, the genotypes and the haplotypes identified for the ICAM2 gene in a reference population. In a preferred embodiment, the computer system is capable of producing a display showing ICAM2 haplotypes organized according to their evolutionary relationships.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a reference sequence for the ICAM2 gene (Genaissance Reference No. 1396765; contiguous lines; SEQ ID NO:1), with the start and stop positions of each region of coding sequence indicated with a bracket ([or]) and the numerical position below the sequence and the polymorphic site(s) and polymorphism(s) identified by Applicants in a reference population indicated by the variant nucleotide positioned below the polymorphic site in the sequence. SEQ ID NO:59 is equivalent to Figure 1, with the two alternative allelic variants of each polymorphic site indicated by the appropriate nucleotide symbol (R= G or A, Y= T or C, M= A or C, K= G or T, S= G or C, and W= A or T; WIPO standard ST.25).

Figure 2 illustrates a reference sequence for the ICAM2 coding sequence (contiguous lines; SEQ ID NO:2), with the polymorphic site(s) and polymorphism(s) identified by Applicants in a reference population indicated by the variant nucleotide positioned below the polymorphic site in the sequence.

Figure 3 illustrates a reference sequence for the ICAM2 protein (contiguous lines; SEQ ID NO:3), with the variant amino acid(s) caused by the polymorphism(s) of Figure 2 positioned below the polymorphic site in the sequence.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based on the discovery of novel variants of the ICAM2 gene. As described in more detail below, the inventors herein discovered 14 isogenes of the ICAM2 gene by characterizing the ICAM2 gene found in genomic DNAs isolated from an Index Repository that contains immortalized cell lines from one chimpanzee and 93 human individuals. The human individuals included a reference population of 79 unrelated individuals self-identified as belonging to one of four major population groups: Caucasian (21 individuals), African descent (20 individuals), Asian (20 individuals), or Hispanic/Latino (18 individuals). To the extent possible, the members of this reference

population were organized into population subgroups by the self-identified ethnogeographic origin as shown in Table 1 below.

Table 1. Population Groups in the Index Repository

Population Group	Population Subgroup	No. of Individuals
African descent		20
	Sierra Leone	1
Asian		20
	Burma	1
	China	3
	Japan	6
	Korea	1
	Philippines	5
	Vietnam	4
Caucasian		21
	British Isles	3
	British Isles/Central	4
	British Isles/Eastern	1
	Central/Eastern	1
	Eastern	3
	Central/Mediterranean	1
	Mediterranean	2
	Scandinavian	2
Hispanic/Latino		18
	Caribbean	8
	Caribbean (Spanish Descent)	2
	Central American (Spanish Descent)	1
	Mexican American	4
	South American (Spanish Descent)	3

In addition, the Index Repository contains three unrelated indigenous American Indians (one from each of North, Central and South America), one three-generation Caucasian family (from the CEPH Utah cohort) and one two-generation African-American family.

The ICAM2 isogenes present in the human reference population are defined by haplotypes for 12 polymorphic sites in the ICAM2 gene, 11 of which are believed to be novel. The ICAM2 polymorphic sites identified by the inventors are referred to as PS1-12 to designate the order in which they are located in the gene (see Table 3 below), with the novel polymorphic sites referred to as PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, and PS11. Using the genotypes identified in the Index Repository for PS1-12 and the methodology described in the Examples below, the inventors herein also determined the pair of haplotypes for the ICAM2 gene present in individual human members of this repository. The human genotypes and haplotypes found in the repository for the ICAM2 gene include those shown in Tables 4 and 5, respectively. The polymorphism and haplotype data disclosed herein are useful for validating whether ICAM2 is a suitable target for drugs to treat HIV infection and inflammatory diseases, screening for such drugs and reducing bias in clinical trials of such drugs.

In the context of this disclosure, the following terms shall be defined as follows unless otherwise

indicated:

Allele - A particular form of a genetic locus, distinguished from other forms by its particular nucleotide sequence.

Candidate Gene - A gene which is hypothesized to be responsible for a disease, condition, or the response to a treatment, or to be correlated with one of these.

Gene - A segment of DNA that contains all the information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

Genotype - An unphased 5' to 3' sequence of nucleotide pair(s) found at one or more polymorphic sites in a locus on a pair of homologous chromosomes in an individual. As used herein, genotype includes a full-genotype and/or a sub-genotype as described below.

Full-genotype - The unphased 5' to 3' sequence of nucleotide pairs found at all known polymorphic sites examined herein in a locus on a pair of homologous chromosomes in a single individual.

Sub-genotype - The unphased 5' to 3' sequence of nucleotides seen at a subset of the known polymorphic sites examined herein in a locus on a pair of homologous chromosomes in a single individual.

Genotyping - A process for determining a genotype of an individual.

Haplotype - A 5' to 3' sequence of nucleotides found at one or more polymorphic sites in a locus on a single chromosome from a single individual. As used herein, haplotype includes a full-haplotype and/or a sub-haplotype as described below.

Full-haplotype - The 5' to 3' sequence of nucleotides found at all known polymorphic sites examined herein in a locus on a single chromosome from a single individual.

Sub-haplotype - The 5' to 3' sequence of nucleotides seen at a subset of the known polymorphic sites examined herein in a locus on a single chromosome from a single individual.

Haplotype pair - The two haplotypes found for a locus in a single individual.

Haplotyping - A process for determining one or more haplotypes in an individual and includes use of family pedigrees, molecular techniques and/or statistical inference.

Haplotype data - Information concerning one or more of the following for a specific gene: a listing of the haplotype pairs in each individual in a population; a listing of the different haplotypes in a population; frequency of each haplotype in that or other populations, and any known associations between one or more haplotypes and a trait.

Isoform - A particular form of a gene, mRNA, cDNA or the protein encoded thereby, distinguished from other forms by its particular sequence and/or structure.

Isogene - One of the isoforms of a gene found in a population. An isogene contains all of the polymorphisms present in the particular isoform of the gene.

Isolated - As applied to a biological molecule such as RNA, DNA, oligonucleotide, or protein,

isolated means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with the methods of the present invention.

Locus - A location on a chromosome or DNA molecule corresponding to a gene or a physical or phenotypic feature.

Naturally-occurring - A term used to designate that the object it is applied to, e.g., naturally-occurring polynucleotide or polypeptide, can be isolated from a source in nature and which has not been intentionally modified by man.

Nucleotide pair - The nucleotides found at a polymorphic site on the two copies of a chromosome from an individual.

Phased - As applied to a sequence of nucleotide pairs for two or more polymorphic sites in a locus, phased means the combination of nucleotides present at those polymorphic sites on a single copy of the locus is known.

Polymorphic site (PS) - A position within a locus at which at least two alternative sequences are found in a population, the most frequent of which has a frequency of no more than 99%.

Polymorphic variant - A gene, mRNA, cDNA, polypeptide or peptide whose nucleotide or amino acid sequence varies from a reference sequence due to the presence of a polymorphism in the gene.

Polymorphism - The sequence variation observed in an individual at a polymorphic site. Polymorphisms include nucleotide substitutions, insertions, deletions and microsatellites and may, but need not, result in detectable differences in gene expression or protein function.

Polymorphism data - Information concerning one or more of the following for a specific gene: location of polymorphic sites; sequence variation at those sites; frequency of polymorphisms in one or more populations; the different genotypes and/or haplotypes determined for the gene; frequency of one or more of these genotypes and/or haplotypes in one or more populations; any known association(s) between a trait and a genotype or a haplotype for the gene.

Polymorphism Database - A collection of polymorphism data arranged in a systematic or methodical way and capable of being individually accessed by electronic or other means.

Polynucleotide - A nucleic acid molecule comprised of single-stranded RNA or DNA or comprised of complementary, double-stranded DNA.

Population Group - A group of individuals sharing a common ethnogeographic origin.

Reference Population - A group of subjects or individuals who are predicted to be representative of the genetic variation found in the general population. Typically, the reference population represents the genetic variation in the population at a certainty level of at least 85%, preferably at least 90%, more preferably at least 95% and even more preferably at least 99%.

Single Nucleotide Polymorphism (SNP) – Typically, the specific pair of nucleotides observed at a single polymorphic site. In rare cases, three or four nucleotides may be found.

Subject – A human individual whose genotypes or haplotypes or response to treatment or disease state are to be determined.

Treatment – A stimulus administered internally or externally to a subject.

Unphased – As applied to a sequence of nucleotide pairs for two or more polymorphic sites in a locus, unphased means the combination of nucleotides present at those polymorphic sites on a single copy of the locus is not known.

As discussed above, information on the identity of genotypes and haplotypes for the ICAM2 gene of any particular individual as well as the frequency of such genotypes and haplotypes in any particular population of individuals is expected to be useful for a variety of drug discovery and development applications. Thus, the invention also provides compositions and methods for detecting the novel ICAM2 polymorphisms and haplotypes identified herein.

The compositions comprise at least one ICAM2 genotyping oligonucleotide. In one embodiment, an ICAM2 genotyping oligonucleotide is a probe or primer capable of hybridizing to a target region that is located close to, or that contains, one of the novel polymorphic sites described herein. As used herein, the term “oligonucleotide” refers to a polynucleotide molecule having less than about 100 nucleotides. A preferred oligonucleotide of the invention is 10 to 35 nucleotides long. More preferably, the oligonucleotide is between 15 and 30, and most preferably, between 20 and 25 nucleotides in length. The exact length of the oligonucleotide will depend on many factors that are routinely considered and practiced by the skilled artisan. The oligonucleotide may be comprised of any phosphorylation state of ribonucleotides, deoxyribonucleotides, and acyclic nucleotide derivatives, and other functionally equivalent derivatives. Alternatively, oligonucleotides may have a phosphate-free backbone, which may be comprised of linkages such as carboxymethyl, acetamidate, carbamate, polyamide (peptide nucleic acid (PNA)) and the like (Varma, R. in Molecular Biology and Biotechnology, A Comprehensive Desk Reference, Ed. R. Meyers, VCH Publishers, Inc. (1995), pages 617-620). Oligonucleotides of the invention may be prepared by chemical synthesis using any suitable methodology known in the art, or may be derived from a biological sample, for example, by restriction digestion. The oligonucleotides may be labeled, according to any technique known in the art, including use of radiolabels, fluorescent labels, enzymatic labels, proteins, haptens, antibodies, sequence tags and the like.

Genotyping oligonucleotides of the invention must be capable of specifically hybridizing to a target region of an ICAM2 polynucleotide, i.e., an ICAM2 isogene. As used herein, specific hybridization means the oligonucleotide forms an anti-parallel double-stranded structure with the target region under certain hybridizing conditions, while failing to form such a structure when incubated with a non-target region or a non-ICAM2 polynucleotide under the same hybridizing conditions. Preferably, the oligonucleotide specifically hybridizes to the target region under conventional high stringency

conditions. The skilled artisan can readily design and test oligonucleotide probes and primers suitable for detecting polymorphisms in the ICAM2 gene using the polymorphism information provided herein in conjunction with the known sequence information for the ICAM2 gene and routine techniques.

A nucleic acid molecule such as an oligonucleotide or polynucleotide is said to be a "perfect" or "complete" complement of another nucleic acid molecule if every nucleotide of one of the molecules is complementary to the nucleotide at the corresponding position of the other molecule. A nucleic acid molecule is "substantially complementary" to another molecule if it hybridizes to that molecule with sufficient stability to remain in a duplex form under conventional low-stringency conditions. Conventional hybridization conditions are described, for example, by Sambrook J. et al., in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989) and by Haymes, B.D. et al. in *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, D.C. (1985). While perfectly complementary oligonucleotides are preferred for detecting polymorphisms, departures from complete complementarity are contemplated where such departures do not prevent the molecule from specifically hybridizing to the target region. For example, an oligonucleotide primer may have a non-complementary fragment at its 5' end, with the remainder of the primer being complementary to the target region. Alternatively, non-complementary nucleotides may be interspersed into the oligonucleotide probe or primer as long as the resulting probe or primer is still capable of specifically hybridizing to the target region.

Preferred genotyping oligonucleotides of the invention are allele-specific oligonucleotides. As used herein, the term allele-specific oligonucleotide (ASO) means an oligonucleotide that is able, under sufficiently stringent conditions, to hybridize specifically to one allele of a gene, or other locus, at a target region containing a polymorphic site while not hybridizing to the corresponding region in another allele(s). As understood by the skilled artisan, allele-specificity will depend upon a variety of readily optimized stringency conditions, including salt and formamide concentrations, as well as temperatures for both the hybridization and washing steps. Examples of hybridization and washing conditions typically used for ASO probes are found in Kogan et al., "Genetic Prediction of Hemophilia A" in *PCR Protocols, A Guide to Methods and Applications*, Academic Press, 1990 and Ruaño et al., *87 Proc. Natl. Acad. Sci. USA* 6296-6300, 1990. Typically, an ASO will be perfectly complementary to one allele while containing a single mismatch for another allele.

Allele-specific oligonucleotides of the invention include ASO probes and ASO primers. ASO probes which usually provide good discrimination between different alleles are those in which a central position of the oligonucleotide probe aligns with the polymorphic site in the target region (e.g., approximately the 7th or 8th position in a 15mer, the 8th or 9th position in a 16mer, and the 10th or 11th position in a 20mer). An ASO primer of the invention has a 3' terminal nucleotide, or preferably a 3' penultimate nucleotide, that is complementary to only one nucleotide of a particular SNP, thereby acting as a primer for polymerase-mediated extension only if the allele containing that nucleotide is present. ASO probes and primers hybridizing to either the coding or noncoding strand are contemplated by the

invention.

ASO probes and primers listed below use the appropriate nucleotide symbol (R= G or A, Y= T or C, M= A or C, K= G or T, S= G or C, and W= A or T; WIPO standard ST.25) at the position of the polymorphic site to represent the two alternative allelic variants observed at that polymorphic site.

A preferred ASO probe for detecting ICAM2 gene polymorphisms comprises a nucleotide sequence, listed 5' to 3', selected from the group consisting of:

TTGGATGSTTGTCTC (SEQ ID NO:4) and its complement,
 CCTCTTTYGGTTACA (SEQ ID NO:5) and its complement,
 CCTCTTCRCCCTGAT (SEQ ID NO:6) and its complement,
 GGTGCCCCTGTTCCCT (SEQ ID NO:7) and its complement,
 GTCCCCARTGACCTT (SEQ ID NO:8) and its complement,
 TGAGGGGRGATCCGT (SEQ ID NO:9) and its complement,
 GGAGTGAKAAGTCAC (SEQ ID NO:10) and its complement,
 ATAAGTCRCTTTCAG (SEQ ID NO:11) and its complement,
 CTGTGTCRGACAGCC (SEQ ID NO:12) and its complement,
 TAGTCACRGTGGTGT (SEQ ID NO:13) and its complement, and
 ATCTTCGRCCAGCAC (SEQ ID NO:14) and its complement.

A preferred ASO primer for detecting ICAM2 gene polymorphisms comprises a nucleotide sequence, listed 5' to 3', selected from the group consisting of:

TGGCCTTTGGATGST (SEQ ID NO:15); GCCTGGGAGACAASC (SEQ ID NO:16);
 AGATGTCCTCTTTYG (SEQ ID NO:17); GGGTCCTGTAACCRA (SEQ ID NO:18);
 TGTGGCCCTCTTCRC (SEQ ID NO:19); CAGCAGATCAGGGYG (SEQ ID NO:20);
 GGTGGGGGTGCCCCYG (SEQ ID NO:21); AGCCACAGGGAACRG (SEQ ID NO:22);
 AGCGGGGTCCCCART (SEQ ID NO:23); AGAGGAAAGGTCAIT (SEQ ID NO:24);
 CTATGGTGAGGGGRG (SEQ ID NO:25); CCATCCACGGATCYC (SEQ ID NO:26);
 GAGTTGGGAGTGAKA (SEQ ID NO:27); CTGAAAGTGACTTMT (SEQ ID NO:28);
 GGAGTGATAAGTCRC (SEQ ID NO:29); GAGGGGCTGAAAGYG (SEQ ID NO:30);
 CAGAGCCTGTGTCRG (SEQ ID NO:31); CCATCTGGCTGTCTYG (SEQ ID NO:32);
 TCATCATAGTCACRG (SEQ ID NO:33); ACACCGACACCACYG (SEQ ID NO:34);
 TGCTTCATCTTCGRC (SEQ ID NO:35); and GCGCAAGTGCTGGYC (SEQ ID NO:36).

Other genotyping oligonucleotides of the invention hybridize to a target region located one to several nucleotides downstream of one of the novel polymorphic sites identified herein. Such oligonucleotides are useful in polymerase-mediated primer extension methods for detecting one of the novel polymorphisms described herein and therefore such genotyping oligonucleotides are referred to herein as "primer-extension oligonucleotides". In a preferred embodiment, the 3'-terminus of a primer-extension oligonucleotide is a deoxynucleotide complementary to the nucleotide located immediately adjacent to the polymorphic site.

A particularly preferred oligonucleotide primer for detecting ICAM2 gene polymorphisms by primer extension terminates in a nucleotide sequence, listed 5' to 3', selected from the group consisting of:

CCTTTGGATG (SEQ ID NO:37); TGGGAGACAA (SEQ ID NO:38);
 TGTCTCTTTT (SEQ ID NO:39); TCCTGTAACC (SEQ ID NO:40);
 GGCCCTCTTC (SEQ ID NO:41); CAGATCAGGG (SEQ ID NO:42);

GGGGGTGCCC (SEQ ID NO: 43); CACAGGGAAC (SEQ ID NO: 44);
 GGGGTCCCCA (SEQ ID NO: 45); GGAAAGGTCA (SEQ ID NO: 46);
 TGGTGAGGGG (SEQ ID NO: 47); TCCACGGATC (SEQ ID NO: 48);
 TTGGGAGTGA (SEQ ID NO: 49); AAAGTGACTT (SEQ ID NO: 50);
 GTGATAAGTC (SEQ ID NO: 51); GGGCTGAAAG (SEQ ID NO: 52);
 AGCCTGTGTC (SEQ ID NO: 53); TCTGGCTGTC (SEQ ID NO: 54);
 TCATAGTCAC (SEQ ID NO: 55); CCGACACCAC (SEQ ID NO: 56);
 TTCATCTTCG (SEQ ID NO: 57); and CAAGTGCTGG (SEQ ID NO: 58).

In some embodiments, a composition contains two or more differently labeled genotyping oligonucleotides for simultaneously probing the identity of nucleotides at two or more polymorphic sites. It is also contemplated that primer compositions may contain two or more sets of allele-specific primer pairs to allow simultaneous targeting and amplification of two or more regions containing a polymorphic site.

ICAM2 genotyping oligonucleotides of the invention may also be immobilized on or synthesized on a solid surface such as a microchip, bead, or glass slide (see, e.g., WO 98/20020 and WO 98/20019). Such immobilized genotyping oligonucleotides may be used in a variety of polymorphism detection assays, including but not limited to probe hybridization and polymerase extension assays. Immobilized ICAM2 genotyping oligonucleotides of the invention may comprise an ordered array of oligonucleotides designed to rapidly screen a DNA sample for polymorphisms in multiple genes at the same time.

In another embodiment, the invention provides a kit comprising at least two genotyping oligonucleotides packaged in separate containers. The kit may also contain other components such as hybridization buffer (where the oligonucleotides are to be used as a probe) packaged in a separate container. Alternatively, where the oligonucleotides are to be used to amplify a target region, the kit may contain, packaged in separate containers, a polymerase and a reaction buffer optimized for primer extension mediated by the polymerase, such as PCR.

The above described oligonucleotide compositions and kits are useful in methods for genotyping and/or haplotyping the ICAM2 gene in an individual. As used herein, the terms "ICAM2 genotype" and "ICAM2 haplotype" mean the genotype or haplotype contains the nucleotide pair or nucleotide, respectively, that is present at one or more of the novel polymorphic sites described herein and may optionally also include the nucleotide pair or nucleotide present at one or more additional polymorphic sites in the ICAM2 gene. The additional polymorphic sites may be currently known polymorphic sites or sites that are subsequently discovered.

One embodiment of the genotyping method involves isolating from the individual a nucleic acid sample comprising the two copies of the ICAM2 gene, or a fragment thereof, that are present in the individual, and determining the identity of the nucleotide pair at one or more polymorphic sites selected from the group consisting of PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, and PS11 in the two copies to assign an ICAM2 genotype to the individual. As will be readily understood by the skilled artisan, the two "copies" of a gene in an individual may be the same allele or may be different alleles. In

a preferred embodiment of the genotyping method, the identity of the nucleotide pair at PS12 is also determined. In a particularly preferred embodiment, the genotyping method comprises determining the identity of the nucleotide pair at each of PS1-12.

Typically, the nucleic acid sample is isolated from a biological sample taken from the individual, such as a blood sample or tissue sample. Suitable tissue samples include whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. The nucleic acid sample may be comprised of genomic DNA, mRNA, or cDNA and, in the latter two cases, the biological sample must be obtained from a tissue in which the ICAM2 gene is expressed. Furthermore it will be understood by the skilled artisan that mRNA or cDNA preparations would not be used to detect polymorphisms located in introns or in 5' and 3' untranslated regions. If an ICAM2 gene fragment is isolated, it must contain the polymorphic site(s) to be genotyped.

One embodiment of the haplotyping method comprises isolating from the individual a nucleic acid sample containing only one of the two copies of the ICAM2 gene, or a fragment thereof, that is present in the individual and determining in that copy the identity of the nucleotide at one or more polymorphic sites selected from the group consisting of PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, and PS11 in that copy to assign an ICAM2 haplotype to the individual. The nucleic acid may be isolated using any method capable of separating the two copies of the ICAM2 gene or fragment such as one of the methods described above for preparing ICAM2 isogenes, with targeted *in vivo* cloning being the preferred approach. As will be readily appreciated by those skilled in the art, any individual clone will only provide haplotype information on one of the two ICAM2 gene copies present in an individual. If haplotype information is desired for the individual's other copy, additional ICAM2 clones will need to be examined. Typically, at least five clones should be examined to have more than a 90% probability of haplotyping both copies of the ICAM2 gene in an individual. In some embodiments, the haplotyping method also comprises identifying the nucleotide at PS12. In a particularly preferred embodiment, the nucleotide at each of PS1-12 is identified.

In another embodiment, the haplotyping method comprises determining whether an individual has one or more of the ICAM2 haplotypes shown in Table 5. This can be accomplished by identifying, for one or both copies of the individual's ICAM2 gene, the phased sequence of nucleotides present at each of PS1-12. The present invention also contemplates that typically only a subset of PS1-12 will need to be directly examined to assign to an individual one or more of the haplotypes shown in Table 5. This is because at least one polymorphic site in a gene is frequently in strong linkage disequilibrium with one or more other polymorphic sites in that gene (Drysdale, CM et al. 2000 *PNAS* 97:10483-10488; Rieder MJ et al. 1999 *Nature Genetics* 22:59-62). Two sites are said to be in linkage disequilibrium if the presence of a particular variant at one site enhances the predictability of another variant at the second site (Stephens, JC 1999, *Mol. Diag.* 4:309-317). Techniques for determining whether any two polymorphic sites are in linkage disequilibrium are well-known in the art (Weir B.S. 1996 *Genetic Data Analysis II*, Sinauer Associates, Inc. Publishers, Sunderland, MA).

In a preferred embodiment, an ICAM2 haplotype pair is determined for an individual by identifying the phased sequence of nucleotides at one or more polymorphic sites selected from the group consisting of PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, and PS11 in each copy of the ICAM2 gene that is present in the individual. In a particularly preferred embodiment, the haplotyping method comprises identifying the phased sequence of nucleotides at each of PS1-12 in each copy of the ICAM2 gene. When haplotyping both copies of the gene, the identifying step is preferably performed with each copy of the gene being placed in separate containers. However, it is also envisioned that if the two copies are labeled with different tags, or are otherwise separately distinguishable or identifiable, it could be possible in some cases to perform the method in the same container. For example, if first and second copies of the gene are labeled with different first and second fluorescent dyes, respectively, and an allele-specific oligonucleotide labeled with yet a third different fluorescent dye is used to assay the polymorphic site(s), then detecting a combination of the first and third dyes would identify the polymorphism in the first gene copy while detecting a combination of the second and third dyes would identify the polymorphism in the second gene copy.

In both the genotyping and haplotyping methods, the identity of a nucleotide (or nucleotide pair) at a polymorphic site(s) may be determined by amplifying a target region(s) containing the polymorphic site(s) directly from one or both copies of the ICAM2 gene, or a fragment thereof, and the sequence of the amplified region(s) determined by conventional methods. It will be readily appreciated by the skilled artisan that only one nucleotide will be detected at a polymorphic site in individuals who are homozygous at that site, while two different nucleotides will be detected if the individual is heterozygous for that site. The polymorphism may be identified directly, known as positive-type identification, or by inference, referred to as negative-type identification. For example, where a SNP is known to be guanine and cytosine in a reference population, a site may be positively determined to be either guanine or cytosine for an individual homozygous at that site, or both guanine and cytosine, if the individual is heterozygous at that site. Alternatively, the site may be negatively determined to be not guanine (and thus cytosine/cytosine) or not cytosine (and thus guanine/guanine).

The target region(s) may be amplified using any oligonucleotide-directed amplification method, including but not limited to polymerase chain reaction (PCR) (U.S. Patent No. 4,965,188), ligase chain reaction (LCR) (Barany et al., *Proc. Natl. Acad. Sci. USA* 88:189-193, 1991; WO90/01069), and oligonucleotide ligation assay (OLA) (Landegren et al., *Science* 241:1077-1080, 1988).

Other known nucleic acid amplification procedures may be used to amplify the target region including transcription-based amplification systems (U.S. Patent No. 5,130,238; EP 329,822; U.S. Patent No. 5,169,766, WO89/06700) and isothermal methods (Walker et al., *Proc. Natl. Acad. Sci. USA* 89:392-396, 1992).

A polymorphism in the target region may also be assayed before or after amplification using one of several hybridization-based methods known in the art. Typically, allele-specific oligonucleotides are utilized in performing such methods. The allele-specific oligonucleotides may be used as differently

labeled probe pairs, with one member of the pair showing a perfect match to one variant of a target sequence and the other member showing a perfect match to a different variant. In some embodiments, more than one polymorphic site may be detected at once using a set of allele-specific oligonucleotides or oligonucleotide pairs. Preferably, the members of the set have melting temperatures within 5°C, and more preferably within 2°C, of each other when hybridizing to each of the polymorphic sites being detected.

Hybridization of an allele-specific oligonucleotide to a target polynucleotide may be performed with both entities in solution, or such hybridization may be performed when either the oligonucleotide or the target polynucleotide is covalently or noncovalently affixed to a solid support. Attachment may be mediated, for example, by antibody-antigen interactions, poly-L-Lys, streptavidin or avidin-biotin, salt bridges, hydrophobic interactions, chemical linkages, UV cross-linking baking, etc. Allele-specific oligonucleotides may be synthesized directly on the solid support or attached to the solid support subsequent to synthesis. Solid-supports suitable for use in detection methods of the invention include substrates made of silicon, glass, plastic, paper and the like, which may be formed, for example, into wells (as in 96-well plates), slides, sheets, membranes, fibers, chips, dishes, and beads. The solid support may be treated, coated or derivatized to facilitate the immobilization of the allele-specific oligonucleotide or target nucleic acid.

The genotype or haplotype for the ICAM2 gene of an individual may also be determined by hybridization of a nucleic acid sample containing one or both copies of the gene, or fragment(s) thereof, to nucleic acid arrays and subarrays such as described in WO 95/11995. The arrays would contain a battery of allele-specific oligonucleotides representing each of the polymorphic sites to be included in the genotype or haplotype.

The identity of polymorphisms may also be determined using a mismatch detection technique, including but not limited to the RNase protection method using riboprobes (Winter et al., *Proc. Natl. Acad. Sci. USA* 82:7575, 1985; Meyers et al., *Science* 230:1242, 1985) and proteins which recognize nucleotide mismatches, such as the E. coli mutS protein (Modrich, *P. Ann. Rev. Genet.* 25:229-253, 1991). Alternatively, variant alleles can be identified by single strand conformation polymorphism (SSCP) analysis (Orita et al., *Genomics* 5:874-879, 1989; Humphries et al., in *Molecular Diagnosis of Genetic Diseases*, R. Elles, ed., pp. 321-340, 1996) or denaturing gradient gel electrophoresis (DGGE) (Wartell et al., *Nucl. Acids Res.* 18:2699-2706, 1990; Sheffield et al., *Proc. Natl. Acad. Sci. USA* 86:232-236, 1989).

A polymerase-mediated primer extension method may also be used to identify the polymorphism(s). Several such methods have been described in the patent and scientific literature and include the "Genetic Bit Analysis" method (WO92/15712) and the ligase/polymerase mediated genetic bit analysis (U.S. Patent 5,679,524. Related methods are disclosed in WO91/02087, WO90/09455, WO95/17676, U.S. Patent Nos. 5,302,509, and 5,945,283. Extended primers containing a polymorphism may be detected by mass spectrometry as described in U.S. Patent No. 5,605,798.

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Another primer extension method is allele-specific PCR (Ruano et al., *Nucl. Acids Res.* 17:8392, 1989; Ruano et al., *Nucl. Acids Res.* 19, 6877-6882, 1991; WO 93/22456; Turki et al., *J. Clin. Invest.* 95:1635-1641, 1995). In addition, multiple polymorphic sites may be investigated by simultaneously amplifying multiple regions of the nucleic acid using sets of allele-specific primers as described in Wallace et al. (WO89/10414).

In addition, the identity of the allele(s) present at any of the novel polymorphic sites described herein may be indirectly determined by genotyping another polymorphic site that is in linkage disequilibrium with the polymorphic site that is of interest. Polymorphic sites in linkage disequilibrium with the presently disclosed polymorphic sites may be located in regions of the gene or in other genomic regions not examined herein. Genotyping of a polymorphic site in linkage disequilibrium with the novel polymorphic sites described herein may be performed by, but is not limited to, any of the above-mentioned methods for detecting the identity of the allele at a polymorphic site.

In another aspect of the invention, an individual's ICAM2 haplotype pair is predicted from its ICAM2 genotype using information on haplotype pairs known to exist in a reference population. In its broadest embodiment, the haplotyping prediction method comprises identifying an ICAM2 genotype for the individual at two or more ICAM2 polymorphic sites described herein, enumerating all possible haplotype pairs which are consistent with the genotype, accessing data containing ICAM2 haplotype pairs identified in a reference population, and assigning a haplotype pair to the individual that is consistent with the data. In one embodiment, the reference haplotype pairs include the ICAM2 haplotype pairs shown in Table 4.

Generally, the reference population should be composed of randomly-selected individuals representing the major ethnogeographic groups of the world. A preferred reference population for use in the methods of the present invention comprises an approximately equal number of individuals from Caucasian, African American, Asian and Hispanic-Latino population groups with the minimum number of each group being chosen based on how rare a haplotype one wants to be guaranteed to see. For example, if one wants to have a q% chance of not missing a haplotype that exists in the population at a p% frequency of occurring in the reference population, the number of individuals (n) who must be sampled is given by $2n = \log(1-q)/\log(1-p)$ where p and q are expressed as fractions. A preferred reference population allows the detection of any haplotype whose frequency is at least 10% with about 99% certainty and comprises about 20 unrelated individuals from each of the four population groups named above. A particularly preferred reference population includes a 3-generation family representing one or more of the four population groups to serve as controls for checking quality of haplotyping procedures.

In a preferred embodiment, the haplotype frequency data for each ethnogeographic group is examined to determine whether it is consistent with Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium (D.L. Hartl et al., *Principles of Population Genomics*, Sinauer Associates (Sunderland, MA), 3rd Ed., 1997) postulates that the frequency of finding the haplotype pair H_1 / H_2 is equal to

$p_{H-W}(H_1 / H_2) = 2p(H_1)p(H_2)$ if $H_1 \neq H_2$ and $p_{H-W}(H_1 / H_2) = p(H_1)p(H_2)$ if $H_1 = H_2$. A statistically significant difference between the observed and expected haplotype frequencies could be due to one or more factors including significant inbreeding in the population group, strong selective pressure on the gene, sampling bias, and/or errors in the genotyping process. If large deviations from Hardy-Weinberg equilibrium are observed in an ethnogeographic group, the number of individuals in that group can be increased to see if the deviation is due to a sampling bias. If a larger sample size does not reduce the difference between observed and expected haplotype pair frequencies, then one may wish to consider haplotyping the individual using a direct haplotyping method such as, for example, CLASPER System™ technology (U.S. Patent No. 5,866,404), single molecule dilution, or allele-specific long-range PCR (Michalotos-Beloin et al., *Nucleic Acids Res.* 24:4841-4843, 1996).

In one embodiment of this method for predicting an ICAM2 haplotype pair for an individual, the assigning step involves performing the following analysis. First, each of the possible haplotype pairs is compared to the haplotype pairs in the reference population. Generally, only one of the haplotype pairs in the reference population matches a possible haplotype pair and that pair is assigned to the individual. Occasionally, only one haplotype represented in the reference haplotype pairs is consistent with a possible haplotype pair for an individual, and in such cases the individual is assigned a haplotype pair containing this known haplotype and a new haplotype derived by subtracting the known haplotype from the possible haplotype pair. In rare cases, either no haplotypes in the reference population are consistent with the possible haplotype pairs, or alternatively, multiple reference haplotype pairs are consistent with the possible haplotype pairs. In such cases, the individual is preferably haplotyped using a direct molecular haplotyping method such as, for example, CLASPER System™ technology (U.S. Patent No. 5,866,404), SMD, or allele-specific long-range PCR (Michalotos-Beloin et al., *supra*). A preferred process for predicting ICAM2 haplotype pairs from ICAM2 genotypes is described in U.S. Provisional Application Serial No. 60/198,340 and the corresponding International Application filed April 18, 2001.

The invention also provides a method for determining the frequency of an ICAM2 genotype, haplotype, or haplotype pair in a population. The method comprises, for each member of the population, determining the genotype or the haplotype pair for the novel ICAM2 polymorphic sites described herein, and calculating the frequency any particular genotype, haplotype, or haplotype pair is found in the population. The population may be a reference population, a family population, a same sex population, a population group, or a trait population (e.g., a group of individuals exhibiting a trait of interest such as a medical condition or response to a therapeutic treatment).

In another aspect of the invention, frequency data for ICAM2 genotypes, haplotypes, and/or haplotype pairs are determined in a reference population and used in a method for identifying an association between a trait and an ICAM2 genotype, haplotype, or haplotype pair. The trait may be any detectable phenotype, including but not limited to susceptibility to a disease or response to a treatment. The method involves obtaining data on the frequency of the genotype(s), haplotype(s), or haplotype pair(s) of interest in a reference population as well as in a population exhibiting the trait. Frequency data

for one or both of the reference and trait populations may be obtained by genotyping or haplotyping each individual in the populations using one of the methods described above. The haplotypes for the trait population may be determined directly or, alternatively, by the predictive genotype to haplotype approach described above. In another embodiment, the frequency data for the reference and/or trait populations is obtained by accessing previously determined frequency data, which may be in written or electronic form. For example, the frequency data may be present in a database that is accessible by a computer. Once the frequency data is obtained, the frequencies of the genotype(s), haplotype(s), or haplotype pair(s) of interest in the reference and trait populations are compared. In a preferred embodiment, the frequencies of all genotypes, haplotypes, and/or haplotype pairs observed in the populations are compared. If a particular ICAM2 genotype, haplotype, or haplotype pair is more frequent in the trait population than in the reference population at a statistically significant amount, then the trait is predicted to be associated with that ICAM2 genotype, haplotype or haplotype pair. Preferably, the ICAM2 genotype, haplotype, or haplotype pair being compared in the trait and reference populations is selected from the full-genotypes and full-haplotypes shown in Tables 4 and 5, or from sub-genotypes and sub-haplotypes derived from these genotypes and haplotypes.

In a preferred embodiment of the method, the trait of interest is a clinical response exhibited by a patient to some therapeutic treatment, for example, response to a drug targeting ICAM2 or response to a therapeutic treatment for a medical condition. As used herein, "medical condition" includes but is not limited to any condition or disease manifested as one or more physical and/or psychological symptoms for which treatment is desirable, and includes previously and newly identified diseases and other disorders. As used herein the term "clinical response" means any or all of the following: a quantitative measure of the response, no response, and adverse response (i.e., side effects).

In order to deduce a correlation between clinical response to a treatment and an ICAM2 genotype, haplotype, or haplotype pair, it is necessary to obtain data on the clinical responses exhibited by a population of individuals who received the treatment, hereinafter the "clinical population". This clinical data may be obtained by analyzing the results of a clinical trial that has already been run and/or the clinical data may be obtained by designing and carrying out one or more new clinical trials. As used herein, the term "clinical trial" means any research study designed to collect clinical data on responses to a particular treatment, and includes but is not limited to phase I, phase II and phase III clinical trials. Standard methods are used to define the patient population and to enroll subjects.

It is preferred that the individuals included in the clinical population have been graded for the existence of the medical condition of interest. This is important in cases where the symptom(s) being presented by the patients can be caused by more than one underlying condition, and where treatment of the underlying conditions are not the same. An example of this would be where patients experience breathing difficulties that are due to either asthma or respiratory infections. If both sets were treated with an asthma medication, there would be a spurious group of apparent non-responders that did not actually have asthma. These people would affect the ability to detect any correlation between haplotype

and treatment outcome. This grading of potential patients could employ a standard physical exam or one or more lab tests. Alternatively, grading of patients could use haplotyping for situations where there is a strong correlation between haplotype pair and disease susceptibility or severity.

The therapeutic treatment of interest is administered to each individual in the trial population and each individual's response to the treatment is measured using one or more predetermined criteria. It is contemplated that in many cases, the trial population will exhibit a range of responses and that the investigator will choose the number of responder groups (e.g., low, medium, high) made up by the various responses. In addition, the ICAM2 gene for each individual in the trial population is genotyped and/or haplotyped, which may be done before or after administering the treatment.

After both the clinical and polymorphism data have been obtained, correlations between individual response and ICAM2 genotype or haplotype content are created. Correlations may be produced in several ways. In one method, individuals are grouped by their ICAM2 genotype or haplotype (or haplotype pair) (also referred to as a polymorphism group), and then the averages and standard deviations of clinical responses exhibited by the members of each polymorphism group are calculated.

These results are then analyzed to determine if any observed variation in clinical response between polymorphism groups is statistically significant. Statistical analysis methods which may be used are described in L.D. Fisher and G. vanBelle, "Biostatistics: A Methodology for the Health Sciences", Wiley-Interscience (New York) 1993. This analysis may also include a regression calculation of which polymorphic sites in the ICAM2 gene give the most significant contribution to the differences in phenotype. One regression model useful in the invention is described in PCT Application Serial No. PCT/US00/17540, entitled "Methods for Obtaining and Using Haplotype Data".

A second method for finding correlations between ICAM2 haplotype content and clinical responses uses predictive models based on error-minimizing optimization algorithms. One of many possible optimization algorithms is a genetic algorithm (R. Judson, "Genetic Algorithms and Their Uses in Chemistry" in Reviews in Computational Chemistry, Vol. 10, pp. 1-73, K. B. Lipkowitz and D. B. Boyd, eds. (VCH Publishers, New York, 1997). Simulated annealing (Press et al., "Numerical Recipes in C: The Art of Scientific Computing", Cambridge University Press (Cambridge) 1992, Ch. 10), neural networks (E. Rich and K. Knight, "Artificial Intelligence", 2nd Edition (McGraw-Hill, New York, 1991, Ch. 18), standard gradient descent methods (Press et al., *supra*, Ch. 10), or other global or local optimization approaches (see discussion in Judson, *supra*) could also be used. Preferably, the correlation is found using a genetic algorithm approach as described in PCT Application Serial No. PCT/US00/17540.

Correlations may also be analyzed using analysis of variation (ANOVA) techniques to determine how much of the variation in the clinical data is explained by different subsets of the polymorphic sites in the ICAM2 gene. As described in PCT Application Serial No. PCT/US00/17540, ANOVA is used to test hypotheses about whether a response variable is caused by or correlated with one

or more traits or variables that can be measured (Fisher and vanBelle, *supra*, Ch. 10).

From the analyses described above, a mathematical model may be readily constructed by the skilled artisan that predicts clinical response as a function of ICAM2 genotype or haplotype content. Preferably, the model is validated in one or more follow-up clinical trials designed to test the model.

The identification of an association between a clinical response and a genotype or haplotype (or haplotype pair) for the ICAM2 gene may be the basis for designing a diagnostic method to determine those individuals who will or will not respond to the treatment, or alternatively, will respond at a lower level and thus may require more treatment, i.e., a greater dose of a drug. The diagnostic method may take one of several forms: for example, a direct DNA test (i.e., genotyping or haplotyping one or more of the polymorphic sites in the ICAM2 gene), a serological test, or a physical exam measurement. The only requirement is that there be a good correlation between the diagnostic test results and the underlying ICAM2 genotype or haplotype that is in turn correlated with the clinical response. In a preferred embodiment, this diagnostic method uses the predictive haplotyping method described above.

In another embodiment, the invention provides an isolated polynucleotide comprising a polymorphic variant of the ICAM2 gene or a fragment of the gene which contains at least one of the novel polymorphic sites described herein. The nucleotide sequence of a variant ICAM2 gene is identical to the reference genomic sequence for those portions of the gene examined, as described in the Examples below, except that it comprises a different nucleotide at one or more of the novel polymorphic sites PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9, PS10, and PS11, and may also comprise an additional polymorphism of adenine at PS12. Similarly, the nucleotide sequence of a variant fragment of the ICAM2 gene is identical to the corresponding portion of the reference sequence except for having a different nucleotide at one or more of the novel polymorphic sites described herein. Thus, the invention specifically does not include polynucleotides comprising a nucleotide sequence identical to the reference sequence of the ICAM2 gene, which is defined by haplotype 3, (or other reported ICAM2 sequences) or to portions of the reference sequence (or other reported ICAM2 sequences), except for genotyping oligonucleotides as described above.

The location of a polymorphism in a variant gene or fragment is identified by aligning its sequence against SEQ ID NO:1. The polymorphism is selected from the group consisting of guanine at PS1, thymine at PS2, guanine at PS3, cytosine at PS4, guanine at PS5, adenine at PS6, guanine at PS7, guanine at PS8, adenine at PS9, adenine at PS10, and adenine at PS11. In a preferred embodiment, the polymorphic variant comprises a naturally-occurring isogene of the ICAM2 gene which is defined by any one of haplotypes 1- 2 and 4 - 14 shown in Table 5 below.

Polymorphic variants of the invention may be prepared by isolating a clone containing the ICAM2 gene from a human genomic library. The clone may be sequenced to determine the identity of the nucleotides at the novel polymorphic sites described herein. Any particular variant claimed herein could be prepared from this clone by performing *in vitro* mutagenesis using procedures well-known in the art.

ICAM2 isogenes may be isolated using any method that allows separation of the two "copies" of the ICAM2 gene present in an individual, which, as readily understood by the skilled artisan, may be the same allele or different alleles. Separation methods include targeted *in vivo* cloning (TIVC) in yeast as described in WO 98/01573, U.S. Patent No. 5,866,404, and U.S. Patent No. 5,972,614. Another method, which is described in U.S. Patent No. 5,972,614, uses an allele specific oligonucleotide in combination with primer extension and exonuclease degradation to generate hemizygous DNA targets. Yet other methods are single molecule dilution (SMD) as described in Ruaño et al., *Proc. Natl. Acad. Sci.* 87:6296-6300, 1990; and allele specific PCR (Ruaño et al., 1989, *supra*; Ruaño et al., 1991, *supra*; Michalatos-Beloin et al., *supra*).

The invention also provides ICAM2 genome anthologies, which are collections of ICAM2 isogenes found in a given population. The population may be any group of at least two individuals, including but not limited to a reference population, a population group, a family population, a clinical population, and a same sex population. An ICAM2 genome anthology may comprise individual ICAM2 isogenes stored in separate containers such as microtest tubes, separate wells of a microtitre plate and the like. Alternatively, two or more groups of the ICAM2 isogenes in the anthology may be stored in separate containers. Individual isogenes or groups of isogenes in a genome anthology may be stored in any convenient and stable form, including but not limited to in buffered solutions, as DNA precipitates, freeze-dried preparations and the like. A preferred ICAM2 genome anthology of the invention comprises a set of isogenes defined by the haplotypes shown in Table 5 below.

An isolated polynucleotide containing a polymorphic variant nucleotide sequence of the invention may be operably linked to one or more expression regulatory elements in a recombinant expression vector capable of being propagated and expressing the encoded ICAM2 protein in a prokaryotic or a eukaryotic host cell. Examples of expression regulatory elements which may be used include, but are not limited to, the lac system, operator and promoter regions of phage lambda, yeast promoters, and promoters derived from vaccinia virus, adenovirus, retroviruses, or SV40. Other regulatory elements include, but are not limited to, appropriate leader sequences, termination codons, polyadenylation signals, and other sequences required for the appropriate transcription and subsequent translation of the nucleic acid sequence in a given host cell. Of course, the correct combinations of expression regulatory elements will depend on the host system used. In addition, it is understood that the expression vector contains any additional elements necessary for its transfer to and subsequent replication in the host cell. Examples of such elements include, but are not limited to, origins of replication and selectable markers. Such expression vectors are commercially available or are readily constructed using methods known to those in the art (e.g., F. Ausubel et al., 1987, in "Current Protocols in Molecular Biology", John Wiley and Sons, New York, New York). Host cells which may be used to express the variant ICAM2 sequences of the invention include, but are not limited to, eukaryotic and mammalian cells, such as animal, plant, insect and yeast cells, and prokaryotic cells, such as *E. coli*, or algal cells as known in the art. The recombinant expression vector may be introduced into the host cell

using any method known to those in the art including, but not limited to, microinjection, electroporation, particle bombardment, transduction, and transfection using DEAE-dextran, lipofection, or calcium phosphate (see e.g., Sambrook et al. (1989) in "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Press, Plainview, New York). In a preferred aspect, eukaryotic expression vectors that function in eukaryotic cells, and preferably mammalian cells, are used. Non-limiting examples of such vectors include vaccinia virus vectors, adenovirus vectors, herpes virus vectors, and baculovirus transfer vectors. Preferred eukaryotic cell lines include COS cells, CHO cells, HeLa cells, NIH/3T3 cells, and embryonic stem cells (Thomson, J. A. et al., 1998 *Science* 282:1145-1147). Particularly preferred host cells are mammalian cells.

As will be readily recognized by the skilled artisan, expression of polymorphic variants of the ICAM2 gene will produce ICAM2 mRNAs varying from each other at any polymorphic site retained in the spliced and processed mRNA molecules. These mRNAs can be used for the preparation of an ICAM2 cDNA comprising a nucleotide sequence which is a polymorphic variant of the ICAM2 reference coding sequence shown in Figure 2. Thus, the invention also provides ICAM2 mRNAs and corresponding cDNAs which comprise a nucleotide sequence that is identical to SEQ ID NO:2 (Fig. 2), or its corresponding RNA sequence, except for having one or more polymorphisms selected from the group consisting of thymine at a position corresponding to nucleotide 12, guanine at a position corresponding to nucleotide 43, adenine at a position corresponding to nucleotide 660, adenine at a position corresponding to nucleotide 687, and adenine at a position corresponding to nucleotide 746, and may also comprise an additional polymorphism of adenine at a position corresponding to nucleotide 822. A particularly preferred polymorphic cDNA variant comprises the coding sequence of an ICAM2 isogene defined by haplotypes 1- 2 and 4 - 14. Fragments of these variant mRNAs and cDNAs are included in the scope of the invention, provided they contain the novel polymorphisms described herein. The invention specifically excludes polynucleotides identical to previously identified and characterized ICAM2 cDNAs and fragments thereof. Polynucleotides comprising a variant RNA or DNA sequence may be isolated from a biological sample using well-known molecular biological procedures or may be chemically synthesized.

As used herein, a polymorphic variant of an ICAM2 gene fragment comprises at least one novel polymorphism identified herein and has a length of at least 10 nucleotides and may range up to the full length of the gene. Preferably, such fragments are between 100 and 3000 nucleotides in length, and more preferably between 200 and 2000 nucleotides in length, and most preferably between 500 and 1000 nucleotides in length.

In describing the ICAM2 polymorphic sites identified herein, reference is made to the sense strand of the gene for convenience. However, as recognized by the skilled artisan, nucleic acid molecules containing the ICAM2 gene may be complementary double stranded molecules and thus reference to a particular site on the sense strand refers as well to the corresponding site on the complementary antisense strand. Thus, reference may be made to the same polymorphic site on either

strand and an oligonucleotide may be designed to hybridize specifically to either strand at a target region containing the polymorphic site. Thus, the invention also includes single-stranded polynucleotides which are complementary to the sense strand of the ICAM2 genomic variants described herein.

Polynucleotides comprising a polymorphic gene variant or fragment may be useful for therapeutic purposes. For example, where a patient could benefit from expression, or increased expression, of a particular ICAM2 protein isoform, an expression vector encoding the isoform may be administered to the patient. The patient may be one who lacks the ICAM2 isogene encoding that isoform or may already have at least one copy of that isogene.

In other situations, it may be desirable to decrease or block expression of a particular ICAM2 isogene. Expression of an ICAM2 isogene may be turned off by transforming a targeted organ, tissue or cell population with an expression vector that expresses high levels of untranslatable mRNA for the isogene. Alternatively, oligonucleotides directed against the regulatory regions (e.g., promoter, introns, enhancers, 3' untranslated region) of the isogene may block transcription. Oligonucleotides targeting the transcription initiation site, e.g., between positions -10 and +10 from the start site are preferred. Similarly, inhibition of transcription can be achieved using oligonucleotides that base-pair with region(s) of the isogene DNA to form triplex DNA (see e.g., Gee et al. in Huber, B.E. and B.I. Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, N.Y., 1994). Antisense oligonucleotides may also be designed to block translation of ICAM2 mRNA transcribed from a particular isogene. It is also contemplated that ribozymes may be designed that can catalyze the specific cleavage of ICAM2 mRNA transcribed from a particular isogene.

The oligonucleotides may be delivered to a target cell or tissue by expression from a vector introduced into the cell or tissue *in vivo* or *ex vivo*. Alternatively, the oligonucleotides may be formulated as a pharmaceutical composition for administration to the patient. Oligoribonucleotides and/or oligodeoxynucleotides intended for use as antisense oligonucleotides may be modified to increase stability and half-life. Possible modifications include, but are not limited to phosphorothioate or 2' O-methyl linkages, and the inclusion of nontraditional bases such as inosine and queosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytosine, guanine, thymine, and uracil which are not as easily recognized by endogenous nucleases.

The invention also provides an isolated polypeptide comprising a polymorphic variant of the reference ICAM2 amino acid sequence shown in Figure 3. The location of a variant amino acid in an ICAM2 polypeptide or fragment of the invention is identified by aligning its sequence against SEQ ID NO:3 (Fig. 3). An ICAM2 protein variant of the invention comprises an amino acid sequence identical to SEQ ID NO:3 except for having one or more variant amino acids selected from the group consisting of alanine at a position corresponding to amino acid position 15 and aspartic acid at a position corresponding to amino acid position 249. The invention specifically excludes amino acid sequences identical to those previously identified for ICAM2, including SEQ ID NO:3, and previously described fragments thereof. ICAM2 protein variants included within the invention comprise all amino acid

sequences based on SEQ ID NO:3 and having the combination of amino acid variations described in Table 2 below. In preferred embodiments, an ICAM2 protein variant of the invention is encoded by an isogene defined by one of the observed haplotypes shown in Table 5.

Table 2. Novel Polymorphic Variants of ICAM2

Polymorphic Variant Number	Amino Acid Position and Identities	
	15	249
1	T	D
2	A	G
3	A	D

The invention also includes ICAM2 peptide variants, which are any fragments of an ICAM2 protein variant that contain one or more of the amino acid variations shown in Table 2. An ICAM2 peptide variant is at least 6 amino acids in length and is preferably any number between 6 and 30 amino acids long, more preferably between 10 and 25, and most preferably between 15 and 20 amino acids long. Such ICAM2 peptide variants may be useful as antigens to generate antibodies specific for one of the above ICAM2 isoforms. In addition, the ICAM2 peptide variants may be useful in drug screening assays.

An ICAM2 variant protein or peptide of the invention may be prepared by chemical synthesis or by expressing one of the variant ICAM2 genomic and cDNA sequences as described above. Alternatively, the ICAM2 protein variant may be isolated from a biological sample of an individual having an ICAM2 isogene which encodes the variant protein. Where the sample contains two different ICAM2 isoforms (i.e., the individual has different ICAM2 isogenes), a particular ICAM2 isoform of the invention can be isolated by immunoaffinity chromatography using an antibody which specifically binds to that particular ICAM2 isoform but does not bind to the other ICAM2 isoform.

The expressed or isolated ICAM2 protein may be detected by methods known in the art, including Coomassie blue staining, silver staining, and Western blot analysis using antibodies specific for the isoform of the ICAM2 protein as discussed further below. ICAM2 variant proteins can be purified by standard protein purification procedures known in the art, including differential precipitation, molecular sieve chromatography, ion-exchange chromatography, isoelectric focusing, gel electrophoresis, affinity and immunoaffinity chromatography and the like. (Ausubel et. al., 1987, In Current Protocols in Molecular Biology John Wiley and Sons, New York, New York). In the case of immunoaffinity chromatography, antibodies specific for a particular polymorphic variant may be used.

A polymorphic variant ICAM2 gene of the invention may also be fused in frame with a heterologous sequence to encode a chimeric ICAM2 protein. The non-ICAM2 portion of the chimeric protein may be recognized by a commercially available antibody. In addition, the chimeric protein may also be engineered to contain a cleavage site located between the ICAM2 and non-ICAM2 portions so that the ICAM2 protein may be cleaved and purified away from the non-ICAM2 portion.

An additional embodiment of the invention relates to using a novel ICAM2 protein isoform in any of a variety of drug screening assays. Such screening assays may be performed to identify agents that bind specifically to all known ICAM2 protein isoforms or to only a subset of one or more of these isoforms. The agents may be from chemical compound libraries, peptide libraries and the like. The ICAM2 protein or peptide variant may be free in solution or affixed to a solid support. In one embodiment, high throughput screening of compounds for binding to an ICAM2 variant may be accomplished using the method described in PCT application WO84/03565, in which large numbers of test compounds are synthesized on a solid substrate, such as plastic pins or some other surface, contacted with the ICAM2 protein(s) of interest and then washed. Bound ICAM2 protein(s) are then detected using methods well-known in the art.

In another embodiment, a novel ICAM2 protein isoform may be used in assays to measure the binding affinities of one or more candidate drugs targeting the ICAM2 protein.

In yet another embodiment, when a particular ICAM2 haplotype or group of ICAM2 haplotypes encodes an ICAM2 protein variant with an amino acid sequence distinct from that of ICAM2 protein isoforms encoded by other ICAM2 haplotypes, then detection of that particular ICAM2 haplotype or group of ICAM2 haplotypes may be accomplished by detecting expression of the encoded ICAM2 protein variant using any of the methods described herein or otherwise commonly known to the skilled artisan.

In another embodiment, the invention provides antibodies specific for and immunoreactive with one or more of the novel ICAM2 variant proteins described herein. The antibodies may be either monoclonal or polyclonal in origin. The ICAM2 protein or peptide variant used to generate the antibodies may be from natural or recombinant sources or produced by chemical synthesis using synthesis techniques known in the art. If the ICAM2 protein variant is of insufficient size to be antigenic, it may be conjugated, complexed, or otherwise covalently linked to a carrier molecule to enhance the antigenicity of the peptide. Examples of carrier molecules, include, but are not limited to, albumins (e.g., human, bovine, fish, ovine), and keyhole limpet hemocyanin (Basic and Clinical Immunology, 1991, Eds. D.P. Stites, and A.I. Terr, Appleton and Lange, Norwalk Connecticut, San Mateo, California).

In one embodiment, an antibody specifically immunoreactive with one of the novel protein isoforms described herein is administered to an individual to neutralize activity of the ICAM2 isoform expressed by that individual. The antibody may be formulated as a pharmaceutical composition which includes a pharmaceutically acceptable carrier.

Antibodies specific for and immunoreactive with one of the novel protein isoforms described herein may be used to immunoprecipitate the ICAM2 protein variant from solution as well as react with ICAM2 protein isoforms on Western or immunoblots of polyacrylamide gels on membrane supports or substrates. In another preferred embodiment, the antibodies will detect ICAM2 protein isoforms in paraffin or frozen tissue sections, or in cells which have been fixed or unfixed and prepared on slides,

coverslips, or the like, for use in immunocytochemical, immunohistochemical, and immunofluorescence techniques.

In another embodiment, an antibody specifically immunoreactive with one of the novel ICAM2 protein variants described herein is used in immunoassays to detect this variant in biological samples. In this method, an antibody of the present invention is contacted with a biological sample and the formation of a complex between the ICAM2 protein variant and the antibody is detected. As described, suitable immunoassays include radioimmunoassay, Western blot assay, immunofluorescent assay, enzyme linked immunoassay (ELISA), chemiluminescent assay, immunohistochemical assay, immunocytochemical assay, and the like (see, e.g., *Principles and Practice of Immunoassay*, 1991, Eds. Christopher P. Price and David J. Neoman, Stockton Press, New York, New York; *Current Protocols in Molecular Biology*, 1987, Eds. Ausubel et al., John Wiley and Sons, New York, New York). Standard techniques known in the art for ELISA are described in *Methods in Immunodiagnosis*, 2nd Ed., Eds. Rose and Bigazzi, John Wiley and Sons, New York 1980; and Campbell et al., 1984, *Methods in Immunology*, W.A. Benjamin, Inc.). Such assays may be direct, indirect, competitive, or noncompetitive as described in the art (see, e.g., *Principles and Practice of Immunoassay*, 1991, Eds. Christopher P. Price and David J. Neoman, Stockton Pres, NY, NY; and Oellirich, M., 1984, *J. Clin. Chem. Clin. Biochem.*, 22:895-904). Proteins may be isolated from test specimens and biological samples by conventional methods, as described in *Current Protocols in Molecular Biology*, supra.

Exemplary antibody molecules for use in the detection and therapy methods of the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules, or those portions of immunoglobulin molecules that contain the antigen binding site. Polyclonal or monoclonal antibodies may be produced by methods conventionally known in the art (e.g., Kohler and Milstein, 1975, *Nature*, 256:495-497; Campbell *Monoclonal Antibody Technology, the Production and Characterization of Rodent and Human Hybridomas*, 1985, In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Eds. Burdon et al., Volume 13, Elsevier Science Publishers, Amsterdam). The antibodies or antigen binding fragments thereof may also be produced by genetic engineering. The technology for expression of both heavy and light chain genes in *E. coli* is the subject of PCT patent applications, publication number WO 901443, WO 901443 and WO 9014424 and in Huse et al., 1989, *Science*, 246:1275-1281. The antibodies may also be humanized (e.g., Queen, C. et al. 1989 *Proc. Natl. Acad. Sci. USA* 86:10029).

Effect(s) of the polymorphisms identified herein on expression of ICAM2 may be investigated by preparing recombinant cells and/or nonhuman recombinant organisms, preferably recombinant animals, containing a polymorphic variant of the ICAM2 gene. As used herein, "expression" includes but is not limited to one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into ICAM2 protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression

and function.

To prepare a recombinant cell of the invention, the desired ICAM2 isogene may be introduced into the cell in a vector such that the isogene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. In a preferred embodiment, the ICAM2 isogene is introduced into a cell in such a way that it recombines with the endogenous ICAM2 gene present in the cell. Such recombination requires the occurrence of a double recombination event, thereby resulting in the desired ICAM2 gene polymorphism. Vectors for the introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector or vector construct may be used in the invention. Methods such as electroporation, particle bombardment, calcium phosphate co-precipitation and viral transduction for introducing DNA into cells are known in the art; therefore, the choice of method may lie with the competence and preference of the skilled practitioner. Examples of cells into which the ICAM2 isogene may be introduced include, but are not limited to, continuous culture cells, such as COS, NIH/3T3, and primary or culture cells of the relevant tissue type, i.e., they express the ICAM2 isogene. Such recombinant cells can be used to compare the biological activities of the different protein variants.

Recombinant nonhuman organisms, i.e., transgenic animals, expressing a variant ICAM2 gene are prepared using standard procedures known in the art. Preferably, a construct comprising the variant gene is introduced into a nonhuman animal or an ancestor of the animal at an embryonic stage, i.e., the one-cell stage, or generally not later than about the eight-cell stage. Transgenic animals carrying the constructs of the invention can be made by several methods known to those having skill in the art. One method involves transfecting into the embryo a retrovirus constructed to contain one or more insulator elements, a gene or genes of interest, and other components known to those skilled in the art to provide a complete shuttle vector harboring the insulated gene(s) as a transgene, see e.g., U.S. Patent No. 5,610,053. Another method involves directly injecting a transgene into the embryo. A third method involves the use of embryonic stem cells. Examples of animals into which the ICAM2 isogenes may be introduced include, but are not limited to, mice, rats, other rodents, and nonhuman primates (see "The Introduction of Foreign Genes into Mice" and the cited references therein, In: Recombinant DNA, Eds. J.D. Watson, M. Gilman, J. Witkowski, and M. Zoller; W.H. Freeman and Company, New York, pages 254-272). Transgenic animals stably expressing a human ICAM2 isogene and producing human ICAM2 protein can be used as biological models for studying diseases related to abnormal ICAM2 expression and/or activity, and for screening and assaying various candidate drugs, compounds, and treatment regimens to reduce the symptoms or effects of these diseases.

An additional embodiment of the invention relates to pharmaceutical compositions for treating disorders affected by expression or function of a novel ICAM2 isogene described herein. The pharmaceutical composition may comprise any of the following active ingredients: a polynucleotide comprising one of these novel ICAM2 isogenes; an antisense oligonucleotide directed against one of the novel ICAM2 isogenes, a polynucleotide encoding such an antisense oligonucleotide, or another

compound which inhibits expression of a novel ICAM2 isogene described herein. Preferably, the composition contains the active ingredient in a therapeutically effective amount. By therapeutically effective amount is meant that one or more of the symptoms relating to disorders affected by expression or function of a novel ICAM2 isogene is reduced and/or eliminated. The composition also comprises a pharmaceutically acceptable carrier, examples of which include, but are not limited to, saline, buffered saline, dextrose, and water. Those skilled in the art may employ a formulation most suitable for the active ingredient, whether it is a polynucleotide, oligonucleotide, protein, peptide or small molecule antagonist. The pharmaceutical composition may be administered alone or in combination with at least one other agent, such as a stabilizing compound. Administration of the pharmaceutical composition may be by any number of routes including, but not limited to oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, intradermal, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

For any composition, determination of the therapeutically effective dose of active ingredient and/or the appropriate route of administration is well within the capability of those skilled in the art. For example, the dose can be estimated initially either in cell culture assays or in animal models. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage will be determined by the practitioner, in light of factors relating to the patient requiring treatment, including but not limited to severity of the disease state, general health, age, weight and gender of the patient, diet, time and frequency of administration, other drugs being taken by the patient, and tolerance/response to the treatment.

Any or all analytical and mathematical operations involved in practicing the methods of the present invention may be implemented by a computer. In addition, the computer may execute a program that generates views (or screens) displayed on a display device and with which the user can interact to view and analyze large amounts of information relating to the ICAM2 gene and its genomic variation, including chromosome location, gene structure, and gene family, gene expression data, polymorphism data, genetic sequence data, and clinical data population data (e.g., data on ethnogeographic origin, clinical responses, genotypes, and haplotypes for one or more populations). The ICAM2 polymorphism data described herein may be stored as part of a relational database (e.g., an instance of an Oracle database or a set of ASCII flat files). These polymorphism data may be stored on the computer's hard drive or may, for example, be stored on a CD-ROM or on one or more other storage devices accessible by the computer. For example, the data may be stored on one or more databases in communication with the computer via a network.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from

consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

EXAMPLES

The Examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the scope of the invention in any way. The Examples do not include detailed descriptions for conventional methods employed, such as in the performance of genomic DNA isolation, PCR and sequencing procedures. Such methods are well-known to those skilled in the art and are described in numerous publications, for example, Sambrook, Fritsch, and Maniatis, "Molecular Cloning: A Laboratory Manual", 2nd Edition, Cold Spring Harbor Laboratory Press, USA, (1989).

EXAMPLE 1

This example illustrates examination of various regions of the ICAM2 gene for polymorphic sites.

Amplification of Target Regions

The following target regions were amplified using the PCR primers represented below. The nucleotide positions of the first and last nucleotide of the forward and reverse primers for each region amplified are presented below and correspond to positions in Figure 1.

PCR Primer Pairs

Fragment No.	Forward Primer	Reverse Primer	PCR Product
Fragment 1	74578-74600	complement of 75200-75181	623 nt
Fragment 2	74893-74916	complement of 75414-75392	522 nt
Fragment 3	76265-76288	complement of 76685-76666	421 nt
Fragment 4	76399-76421	complement of 76903-76880	505 nt
Fragment 5	77649-77668	complement of 78186-78163	538 nt
Fragment 6	77798-77818	complement of 78313-78291	516 nt
Fragment 7	78706-78728	complement of 79227-79205	522 nt

These primer pairs were used in PCR reactions containing genomic DNA isolated from immortalized cell lines for each member of the Index Repository. The PCR reactions were carried out under the following conditions:

Reaction volume	= 10 µl
10 x Advantage 2 Polymerase reaction buffer (Clontech)	= 1 µl
100 ng of human genomic DNA	= 1 µl
10 mM dNTP	= 0.4 µl
Advantage 2 Polymerase enzyme mix (Clontech)	= 0.2 µl
Forward Primer (10 µM)	= 0.4 µl
Reverse Primer (10 µM)	= 0.4 µl
Water	= 6.6µl

Amplification profile:

97°C - 2 min.	1 cycle	
97°C - 15 sec.	} 10 cycles	
70°C - 45 sec.		
72°C - 45 sec.		
97°C - 15 sec.	} 35 cycles	
64°C - 45 sec.		
72°C - 45 sec.		

Sequencing of PCR Products

The PCR products were purified using a Whatman/Polyfiltronics 100 µl 384 well unfilter plate essentially according to the manufacturers protocol. The purified DNA was eluted in 50 µl of distilled water. Sequencing reactions were set up using Applied Biosystems Big Dye Terminator chemistry essentially according to the manufacturers protocol. The purified PCR products were sequenced in both directions using the primer sets represented below with the positions of their first and last nucleotide corresponding to positions in Figure 1. Reaction products were purified by isopropanol precipitation, and run on an Applied Biosystems 3700 DNA Analyzer.

Sequencing Primer Pairs

Fragment No.	Forward Primer	Reverse Primer
Fragment 1	74631-74651	complement of 74083-74063
Fragment 2	74916-74935	complement of 75323-75303
Fragment 3	76292-76311	complement of 76649-76630
Fragment 4	76452-76470	complement of 76821-76802
Fragment 5	77675-77694	complement of 78098-78079
Fragment 6	77855-77874	complement of 78273-78254
Fragment 7	78730-78749	complement of 79163-79143

Analysis of Sequences for Polymorphic Sites

Sequence information for a minimum of 80 humans was analyzed for the presence of polymorphisms using the Polyphred program (Nickerson et al., *Nucleic Acids Res.* 14:2745-2751, 1997). The presence of a polymorphism was confirmed on both strands. The polymorphisms and their locations in the ICAM2 gene are listed in Table 3 below.

Table 3. Polymorphic Sites Identified in the ICAM2 Gene

Polymorphic Site Number	PolyId ^a	Nucleotide Position	Reference Allele	Variant Allele	CDS Position	AA Variant
PS1	8163532	74697	C	G		
PS2	8163530	75110	C	T	12	F4F
PS3	9815754	75141	A	G	43	T15A
PS4	8163528	75221	T	C		
PS5	9813329	76302	A	G		
PS6	8163526	78155	G	A		
PS7	8163524	78181	T	G		
PS8	8163522	78187	A	G		
PS9	8163520	78875	G	A	660	S220S
PS10	8163518	78902	G	A	687	T229T
PS11	8163516	78961	G	A	746	G249D
PS12 ^R	8163513	79037	G	A	822	R274R

^aPolyId is a unique identifier assigned to each PS by Genaissance Pharmaceuticals, Inc.

^RPreviously reported in literature.

EXAMPLE 2

This example illustrates analysis of the ICAM2 polymorphisms identified in the Index Repository for human genotypes and haplotypes.

The different genotypes containing these polymorphisms that were observed in the reference population are shown in Table 4 below, with the haplotype pair indicating the combination of haplotypes determined for the individual using the haplotype derivation protocol described below. In Table 4, homozygous positions are indicated by one nucleotide and heterozygous positions are indicated by two nucleotides. Missing nucleotides in any given genotype in Table 4 were inferred based on linkage disequilibrium and/or Mendelian inheritance.

Table 4(Part1). Genotypes and Haplotype Pairs Observed for ICAM2 Gene

Genotype Number	Polymorphic Sites										HAP	Pair
	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10		
1	G	C	A	C	A	G	G	A	G	G	10	10
2	C	C	A	T	A	G	G	A	G	G	2	2
3	C	C	A	T	A	G	T	A	G	G	3	3
4	G	C	A	C	A	G	G	A	G	G/A	10	7
5	G	C	A	C	A	G	G	A/G	G	G	10	11
6	G/C	C	A	C	A	G	G	A	G/A	G	10	1
7	G	C	A	C	A	G/A	G	A	G	G	10	6
8	G	C	A/G	C	A	G	G	A	G	G	10	16
9	C	C	A	T	A	G	T/G	A	G	G	3	2
10	G/C	C	A	C/T	A	G	G/T	A	G	G	10	3
11	G/C	C	A	C/T	A	G	G	A	G	G	10	2
12	G/C	C/T	A	C/T	A	G	G/T	A	G	G	10	5
13	G	C	A	C	A	G	G	A	G	G	10	9
14	G	C	A	C/T	A	G	G	A	G	G	10	13
15	G/C	C	A	C/T	A/G	G	G/T	A	G	G	10	4
16	G	C	A	C	A/G	G	G	A	G	G	10	12
17	G	C	A	C	A	G	G	A	G	G	10	8
18	C/G	C	A	T/C	A/G	G	T/G	A	G	G	3	12

Table 4(Part2). Genotypes and Haplotype Pairs Observed for ICAM2 Gene

Genotype Number	Polymorphic Sites		HAP Pair	
	PS11	PS12		
1	G	G	10	10
2	G	G	2	2
3	G	G	3	3
4	G	G	10	7
5	G	G	10	11
6	G	G	10	1
7	G	G	10	6
8	G	G	10	16
9	G	G	3	2
10	G	G	10	3
11	G	G	10	2
12	G	G	10	5
13	G	G/A	10	9
14	G	G	10	13
15	G	G/A	10	4
16	G	G/A	10	12
17	G/A	G	10	8
18	G	G/A	3	12

The haplotype pairs shown in Table 4 were estimated from the unphased genotypes using a computer-implemented extension of Clark's algorithm (Clark, A.G. 1990 *Mol Bio Evol* 7, 111-122) for assigning haplotypes to unrelated individuals in a population sample, as described in U.S. Provisional Application Serial No. 60/198,340 entitled "A Method and System for Determining Haplotypes from a Collection of Polymorphisms" and the corresponding International Application filed April 18, 2001. In this method, haplotypes are assigned directly from individuals who are homozygous at all sites or heterozygous at no more than one of the variable sites. This list of haplotypes is augmented with haplotypes obtained from two families (one three-generation Caucasian family and one two-generation African-American family) and then used to deconvolute the unphased genotypes in the remaining (multiply heterozygous) individuals.

By following this protocol, it was determined that the Index Repository examined herein and, by extension, the general population contains the 14 human ICAM2 haplotypes shown in Table 5 below.

Table 5. Haplotypes Identified in the ICAM2 Gene

HAP No.	HAP ID	Polymorphic Sites											
		PS 1	PS 2	PS 3	PS 4	PS 5	PS 6	PS 7	PS 8	PS 9	PS 10	PS 11	PS 12
1	9897355	C	C	A	C	A	G	G	A	A	G	G	G
2	9897352	C	C	A	T	A	G	G	A	G	G	G	G
3	9897351	C	C	A	T	A	G	T	A	G	G	G	G
4	9897357	C	C	A	T	G	G	T	A	G	G	G	A
5	9897356	C	T	A	T	A	G	T	A	G	G	G	G
6	9897364	G	C	A	C	A	A	G	A	G	G	G	G
7	9897362	G	C	A	C	A	G	G	A	G	A	G	G
8	9897361	G	C	A	C	A	G	G	A	G	G	A	G
9	9897365	G	C	A	C	A	G	G	A	G	G	G	A
10	9897350	G	C	A	C	A	G	G	A	G	G	G	G
11	9897363	G	C	A	C	A	G	G	G	G	G	G	G
12	9897353	G	C	A	C	G	G	G	A	G	G	G	A
13	9897359	G	C	A	T	A	G	G	A	G	G	G	G
14	9897360	G	C	G	C	A	G	G	A	G	G	G	G

Isogenes of the ICAM2 gene are identical to the reference sequence at the regions examined except where they vary at the polymorphic site. In particular, a specific ICAM2 isogene identified by a haplotype in Table 5 comprises each oligo in the set of oligos shown in Table 6 for that haplotype.

TTGGATGCTTGTCTC (SEQ ID NO: 60)
 TTGGATGGTTGTCTC (SEQ ID NO: 61)
 CCTCTTTCGGTTACA (SEQ ID NO: 62)
 CCTCTTTTGGTTACA (SEQ ID NO: 63)
 CCTCTTCACCCTGAT (SEQ ID NO: 64)
 CCTCTTCGCCCTGAT (SEQ ID NO: 65)
 GGTGCCCTGTTCCCT (SEQ ID NO: 66)
 GGTGCCCCGTTCCCT (SEQ ID NO: 67)
 GTCCCCAATGACCTT (SEQ ID NO: 68)
 GTCCCCAGTGACCTT (SEQ ID NO: 69)
 TGAGGGGGGATCCGT (SEQ ID NO: 70)
 TGAGGGGAGATCCGT (SEQ ID NO: 71)
 GGAGTGATAAGTCAC (SEQ ID NO: 72)
 GGAGTGAGAAGTCAC (SEQ ID NO: 73)
 ATAAGTCACTTTCAG (SEQ ID NO: 74)
 ATAAGTCGCTTTCAG (SEQ ID NO: 75)
 CTGTGTCGGACAGCC (SEQ ID NO: 76)
 CTGTGTCAGACAGCC (SEQ ID NO: 77)
 TAGTCACGGTGGTGT (SEQ ID NO: 78)
 TAGTCACAGTGGTGT (SEQ ID NO: 79)
 ATCTTCGGCCAGCAC (SEQ ID NO: 80)
 ATCTTCGACCAGCAC (SEQ ID NO: 81)
 CCTTCCGGCCATAGC (SEQ ID NO: 82)
 CCTTCCGACCATAGC (SEQ ID NO: 83)

Table 6 (part 1). Oligonucleotides Comprising Haplotypes for the ICAM2 Gene												
Haplotype Number	PS1		PS2		PS3		PS4		PS5		PS6	
	SEQ ID NO:60	SEQ ID NO:61	SEQ ID NO:62	SEQ ID NO:63	SEQ ID NO:64	SEQ ID NO:65	SEQ ID NO:66	SEQ ID NO:67	SEQ ID NO:68	SEQ ID NO:69	SEQ ID NO:70	SEQ ID NO:71
1	X		X		X			X	X		X	
2	X		X		X		X		X		X	
3	X		X		X		X		X		X	
4	X		X		X		X			X	X	
5	X			X	X		X		X		X	
6		X	X		X			X	X			X
7		X	X		X			X	X		X	
8		X	X		X			X	X		X	
9		X	X		X			X	X		X	
10		X	X		X			X	X		X	
11		X	X		X			X	X		X	
12		X	X		X			X		X	X	
13		X	X		X		X		X		X	
14		X	X			X		X	X		X	

Table 6 (part 2). Oligonucleotides Comprising Haplotypes for the ICAM2 Gene												
Haplotype Number	PS7		PS8		PS9		PS10		PS11		PS12	
	SEQ ID NO:72	SEQ ID NO:73	SEQ ID NO:74	SEQ ID NO:75	SEQ ID NO:76	SEQ ID NO:77	SEQ ID NO:78	SEQ ID NO:79	SEQ ID NO:80	SEQ ID NO:81	SEQ ID NO:82	SEQ ID NO:83
1		X	X			X	X		X		X	
2		X	X		X		X		X		X	
3	X		X		X		X		X		X	
4	X		X		X		X		X			X
5	X		X		X		X		X		X	
6		X	X		X		X		X		X	
7		X	X		X			X	X		X	
8		X	X		X		X			X	X	
9		X	X		X		X		X			X
10		X	X		X		X		X		X	
11		X		X	X		X		X		X	
12		X	X		X		X		X			X
13		X	X		X		X		X		X	
14		X	X		X		X		X		X	

Polymorphic variants of ICAM2 coding sequence isogenes are identical at the regions examined to the reference ICAM2 coding sequence, SEQ ID NO:2 (Fig. 2), except where they vary at a polymorphic site. ICAM2 coding sequence isogenes are defined by subhaplotypes of the full ICAM2 haplotypes shown in Table 6. In particular, a specific ICAM2 coding sequence isogene identified by a sub-haplotype of a haplotype in Table 6 comprises each oligo in the set of oligos shown in Table 7 for that ICAM2 sub-haplotype.

Table 7. Oligonucleotides Comprising Coding Sequence Sub-Haplotypes for the ICAM2 Gene												
Coding Sequence Sub-Haplotype Number	PS2		PS3		PS9		PS10		PS11		PS12	
	SEQ ID NO:62	SEQ ID NO:63	SEQ ID NO:64	SEQ ID NO:65	SEQ ID NO:76	SEQ ID NO:77	SEQ ID NO:78	SEQ ID NO:79	SEQ ID NO:80	SEQ ID NO:81	SEQ ID NO:82	SEQ ID NO:83
1c	X		X			X	X		X		X	
2c	X		X		X		X		X		X	
3c	X		X		X		X		X		X	
4c	X		X		X		X		X			X
5c		X	X		X		X		X		X	
6c	X		X		X		X		X		X	
7c	X		X		X			X	X		X	
8c	X		X		X		X			X	X	
9c	X		X		X		X		X			X
10c	X		X		X		X		X		X	
11c	X		X		X		X		X		X	
12c	X		X		X		X		X			X
13c	X		X		X		X		X		X	
14c	X			X	X		X		X		X	

Table 8 below shows the percent of chromosomes characterized by a given ICAM2 haplotype for all unrelated individuals in the Index Repository for which haplotype data was obtained. The percent of these unrelated individuals who have a given ICAM2 haplotype pair is shown in Table 9. In Tables 8 and 9, the "Total" column shows this frequency data for all of these unrelated individuals, while the other columns show the frequency data for these unrelated individuals categorized according to their self-identified ethnogeographic origin. Abbreviations used in Tables 8 and 9 are AF = African Descent, AS = Asian, CA = Caucasian, HL = Hispanic-Latino, and NA = Native American.

Table 8. Frequency of Observed ICAM2 Haplotypes In Unrelated Individuals

HAP No.	HAP ID	Total	CA	AF	AS	HL	AM
1	9897355	0.61	0.0	2.5	0.0	0.0	0.0
2	9897352	2.44	0.0	10.0	0.0	0.0	0.0
3	9897351	4.88	0.0	20.0	0.0	0.0	0.0
4	9897357	0.61	0.0	2.5	0.0	0.0	0.0
5	9897356	0.61	0.0	2.5	0.0	0.0	0.0
6	9897364	0.61	0.0	0.0	2.5	0.0	0.0
7	9897362	0.61	0.0	0.0	0.0	2.78	0.0
8	9897361	0.61	0.0	0.0	2.5	0.0	0.0
9	9897365	0.61	0.0	2.5	0.0	0.0	0.0
10	9897350	85.37	97.62	50.0	95.0	97.22	100.0
11	9897363	0.61	0.0	2.5	0.0	0.0	0.0
12	9897353	1.22	0.0	5.0	0.0	0.0	0.0
13	9897359	0.61	0.0	2.5	0.0	0.0	0.0
14	9897360	0.61	2.38	0.0	0.0	0.0	0.0

Table 9. Frequency of Observed ICAM2 Haplotype Pairs In Unrelated Individuals

HAP1	HAP2	Total	CA	AF	AS	HL	AM
10	10	75.61	95.24	20.0	90.0	94.44	100.0
2	2	1.22	0.0	5.0	0.0	0.0	0.0
3	3	1.22	0.0	5.0	0.0	0.0	0.0
10	7	1.22	0.0	0.0	0.0	5.56	0.0
10	11	1.22	0.0	5.0	0.0	0.0	0.0
10	1	1.22	0.0	5.0	0.0	0.0	0.0
10	6	1.22	0.0	0.0	5.0	0.0	0.0
10	16	1.22	4.76	0.0	0.0	0.0	0.0
3	2	1.22	0.0	5.0	0.0	0.0	0.0
10	3	4.88	0.0	20.0	0.0	0.0	0.0
10	2	1.22	0.0	5.0	0.0	0.0	0.0
10	5	1.22	0.0	5.0	0.0	0.0	0.0
10	9	1.22	0.0	5.0	0.0	0.0	0.0
10	13	1.22	0.0	5.0	0.0	0.0	0.0
10	4	1.22	0.0	5.0	0.0	0.0	0.0
10	12	1.22	0.0	5.0	0.0	0.0	0.0
10	8	1.22	0.0	0.0	5.0	0.0	0.0
3	12	1.22	0.0	5.0	0.0	0.0	0.0

The size and composition of the Index Repository were chosen to represent the genetic diversity across and within four major population groups comprising the general United States population. For example, as described in Table 1 above, this repository contains approximately equal sample sizes of African-descent, Asian-American, European-American, and Hispanic-Latino population groups. Almost all individuals representing each group had all four grandparents with the same ethnogeographic background. The number of unrelated individuals in the Index Repository provides a sample size that is sufficient to detect SNPs and haplotypes that occur in the general population with high statistical certainty. For instance, a haplotype that occurs with a frequency of 5% in the general population has a probability higher than 99.9% of being observed in a sample of 80 individuals from the general

population. Similarly, a haplotype that occurs with a frequency of 10% in a specific population group has a 99% probability of being observed in a sample of 20 individuals from that population group. In addition, the size and composition of the Index Repository means that the relative frequencies determined therein for the haplotypes and haplotype pairs of the THPO gene are likely to be similar to the relative frequencies of these THPO haplotypes and haplotype pairs in the general U.S. population and in the four population groups represented in the Index Repository. The genetic diversity observed for the three Native Americans is presented because it is of scientific interest, but due to the small sample size it lacks statistical significance.

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification, including patents and patent applications, are hereby incorporated in their entirety by reference. The discussion of references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.